

NICKEL SITE OF METHANE CATALYSIS IN THE METHYL REDUCTASE ENZYME

J. A. Shelnut, A. K. Shiemke,* R. A. Scott*

Sandia National Laboratories, Albuquerque, NM 87185
University of Illinois, Urbana, IL 61801

Introduction

Methyl reductase is the enzyme of methanogenic bacteria that catalyzes the two-electron reduction of the methyl group of 2-(methylthio)ethanesulfonic acid (methyl-S-CoM) to methane and HS-CoM (1,2). The methyl group of methyl-S-CoM ultimately comes from the six-electron reduction of CO₂ by hydrogen, which also provides the reducing equivalents needed by methyl reductase. The nature of the catalytic site of methyl reductase is of current interest from the point of view of developing biomimetic C₁ chemistries directed toward methane synthesis and activation. In particular, Sandia is using molecular graphics and energy optimization techniques to design macromolecular catalysts that mimic the structure of sites of proteins that carry out C₁ chemistry. The goal is to produce catalysts whose function is the oxidation of low molecular weight hydrocarbon gases to generate liquid fuels or, alternatively, the reduction of abundant inorganic resources such as CO₂ to generate gaseous fuels. Unfortunately, the catalytic sites of many of the enzymes of interest, e. g. methyl reductase and methane monooxygenase, have not been characterized by X-ray crystallography and other structural techniques.

With the goal of learning more about the structure of one of these naturally occurring sites of C₁ chemistry, we have obtained the first resonance Raman spectra of the nickel-macrocycle, called F₄₃₀ (1 in Figure 1), at the site of catalysis in methyl reductase (3). To help us structurally interpret the Raman spectra of the enzyme we have also obtained Raman spectra of solutions of the major forms of F₄₃₀ (salt-extracted and cytosol-free) at room temperature and at 77 °K and also, under similar solution conditions, spectra of a nickel-corphinoid derivative (2 in Figure 1) that is related to F₄₃₀ (3-5). By analogy with the spectra of the model nickel-corphinoid 2, the F₄₃₀ Raman spectra characterize the coordination geometry of the nickel(II) ion in F₄₃₀ complexes in coordinating and non-coordinating solvents. In addition, the spectra give some information concerning macrocycle ruffling in the solution complexes. Although some conclusions about the F₄₃₀ site in methyl reductase can be made, the structure of F₄₃₀ in the protein environment is uniquely different from F₄₃₀ and the F₄₃₀ model compound in the solutions we have investigated.

Materials and Methods

Methyl reductase was prepared and purified as described previously (6). F₄₃₀ was isolated in two forms. Free F₄₃₀ was isolated from the cytosol by methods described before (7); F₄₃₀ was also extracted from the holoenzyme using a lithium-bromide procedure (6). The structure 1 of salt-extracted F₄₃₀ has been determined (8) and is shown in Figure 1. A nickel-corphinoid derivative related to F₄₃₀ and shown in 2 of Figure 1 was kindly provided by A. Eschenmoser, A. Pfaltz, and A. Fässler (9).

Samples of each chromophore (2-10x10⁻⁵ M) in aqueous solution buffered with 10 mM phosphate at pH 7 were used for obtaining resonance Raman spectra. Spectra of liquid solutions were obtained using a cylindrical cell partitioned into two compartments. The spectra of two samples were obtained simultaneously by rotating the cell at 100 Hz so that the two sample solutions were alternately probed by the laser radiation. The Raman difference instrumentation used for

detection and separation of the spectra of the two samples has been described previously (10). Peak positions were obtained from the fast-Fourier-transform smoothed spectra. For spectra run simultaneously the accuracy when comparing the frequency of the same Raman line in the two spectra is about $\pm 0.3 \text{ cm}^{-1}$. Occasionally, the solution of F_{430} or the corphinoid derivative was in one side of the cell and the reference side contained only the neat solvent. Subtraction of the solvent Raman lines could then be accurately accomplished leaving the spectrum of only the solute.

Raman spectra at 77 °K were not obtained in the Raman difference mode, but separately using an EPR dewar with a transparent tail (Wilmad). The sample was contained in a 4-mm NMR tube and was frozen by plunging the tube into liquid N_2 . Reproducibility in the frequency of lines in these spectra are about 1 cm^{-1} .

Signal averaged Raman spectra were obtained using the 441.6-nm line of a HeCd laser (Omnichrome) or the 413.6-nm line of a krypton ion laser (Coherent). The spectral resolution was 4 cm^{-1} . Raman difference spectra were obtained using standard 90° scattering geometry; for spectra of frozen solutions a backscattering geometry was used. Absorption spectra as well as individual scans of the Raman spectrum were used to monitor sample integrity. No decomposition of F_{430} or the nickel-corphinoid model was observed.

Results

F_{430} and the model nickel-corphinoid complexes have an absorption band in the 410-440-nm region of the visible spectrum (9,11). Therefore, excitation of the Raman spectrum using 441.6-nm or 413.1-nm laser light is near the visible absorption band and, consequently, resonance enhancement of the Raman scattering occurs. In the case of the enzyme, resonance enhancement of the chromophore's spectrum permits us to selectively probe the macrocyclic cofactor without interference from the Raman spectrum of the protein matrix. Structural details of the F_{430} site in the protein can then be inferred from differences between the spectrum of protein-bound F_{430} and the spectra of F_{430} complexes in various solution environments. The spectra of all of the complexes are somewhat similar regardless of the position of the laser wavelength relative to the absorption band maximum of the particular complex. In fact, the frequencies and intensities of the lines are so similar that the corresponding lines in each of the spectra can easily be identified as can be seen in Figures 2-4. The Figures show typical spectra of salt-extracted F_{430} , cytosol-free F_{430} , and methyl reductase.

Figure 2 shows the Raman spectrum of LiBr-extracted F_{430} at room temperature and at 77 °K. Two forms are present at room temperature. Form A has Raman lines at 1293, 1380, 1534, and (from other Raman data not shown) 1625 cm^{-1} ; the second form B has lines at higher frequencies—1312, 1382, 1555, and 1629 cm^{-1} . At low temperature form A disappears and only B remains.

Free F_{430} at room temperature is predominantly a single form with Raman lines at 1293, 1387, 1529, and 1623 cm^{-1} (Figure 3). The frequencies are similar but distinct from the corresponding lines of the A form of salt-extracted F_{430} . There is also some evidence of a small fraction of a form with lines near those of the B form in the weak shoulders of the high frequency lines. At low temperature this form is more abundant as evidenced by the appearance of lines at 1311, 1378, 1546, and 1616 cm^{-1} that are barely noticeable in the room temperature spectrum. There is also evidence for a second form at low temperature in the lines at 1292 and 1628 cm^{-1} . It is clear that this form is not the same as the one present at room temperature because the 1529-cm^{-1} line is not present or else is shifted to near 1546 cm^{-1} .

The Raman spectrum of methyl reductase at room temperature is markedly different from both the spectrum of salt-extracted F_{430} and free F_{430} . (Compare the spectra in Figure 4 with the corresponding spectra in Figures 2 and 3.) First, the Raman lines are clearly much narrower than for the solution F_{430} complexes. Second, frequencies of the two strong lines at 1575 and 1653 cm^{-1} are much higher than the corresponding lines of the F_{430} species. In contrast, the lines in the 1280-1400- cm^{-1} region of the methyl-reductase spectrum have frequencies comparable to F_{430} .

Differences between room temperature and 77-°K spectra of methyl reductase are much smaller than observed for F_{430} in solution. Increases in the intensity of the lines at 1312, 1553, and 1632 cm^{-1} indicate an enhanced fraction of a form of F_{430} with frequencies closest to those of salt-extracted F_{430} at 77 °K.

Discussion

Raman spectra of the F_{430} forms and the F_{430} model compound 2 are similar. For example, the nickel-corphinoid bis-methanol complex has several weak lines in the 1280-1400- cm^{-1} region and two strong lines at 1556 and 1627 cm^{-1} (4). Thus, the frequency of the lines and the intensities are similar for F_{430} and the model compound. The spectral similarities indicate that the nickel corphinoid 2 is generally a good structural model for F_{430} forms.

In previous work it was noted that the two strong highest frequency lines shift systematically with increasing coordination number (4). For example, the separation between the two lines decreases from 93 cm^{-1} for the 4-coordinate species (in CH_2Cl_2) to 84 cm^{-1} for a 5-coordinate NCS^- complex (in CH_2Cl_2) and to 71 cm^{-1} for the 6-coordinate MeOH complex. In fact, for a variety of solvents that axially ligate, the 6-coordinate separation is found to be 71±3 cm^{-1} (5). Ruffling of the corphinato macrocycle may also influence the separation (vide infra).

Salt-extracted F_{430} . Assuming that the separation of the two strong lines is indicative of coordination number for F_{430} as well as the model, we predict that aqueous salt-extracted F_{430} is a mixture of a dominate 6-coordinate complex referred to above as species B (with a separation of 1629 - 1556 = 73 cm^{-1}) and a minor 4-coordinate species A (with a separation of 1625 - 1534 = 91 cm^{-1}). Indeed, the separation varies from 62-75 cm^{-1} for F_{430} 6-coordinate complexes in other coordinating solvents (5). The existence of a mixture of a 6-coordinate high-spin species and a 4-coordinate S=0 species is supported by the intermediate value found for the magnetic moment (2.0 μ_B for F_{430} versus ~2.8 μ_B predicted for a pure S=1 species) (12).

The axial ligands are probably H_2O molecules rather than some component left bound to F_{430} after extraction from the protein. Water is a weak ligand which would account for the equilibrium mixture at room temperature. At 77 °K the equilibrium shifts overwhelmingly toward the bis-aquo F_{430} complex.

Cytosol-free F_{430} . Water appears to be a poorer ligand for free F_{430} than for the salt-extracted form, since at room temperature aqueous free F_{430} is almost entirely a 4-coordinate form. This conclusion is based on an observed separation of the strong high frequency lines of 94 cm^{-1} —a value close to the separation for the 4-coordinate model compound 2. By comparison, in strongly coordinating solvents and in the presence of CN^- , free F_{430} converts to a 6-coordinate form with a separation of 63-77 cm^{-1} (5).

At 77 °K free F_{430} appears to be a mixture of two forms with lines at 1616 and 1628 cm^{-1} . The separations of the 1616- and 1628- cm^{-1} lines from the 1546- cm^{-1} line are 70 and 82 cm^{-1} for the two forms. EXAFS and XAS measurements at 4 °K are consistent with free F_{430} (the same as heat-extracted F_{430}) existing as a mixture of predominantly a 4-coordinate, ruffled species with short Ni-N bonds (1.9 Å) and a minor 6-coordinate, planar species with longer Ni-N bonds (2.1 Å) (13). The Raman data is in agreement with this result. The species with the 70- cm^{-1} separation is probably the coordinated species and the 82- cm^{-1} separation species would then be the more abundant 4-coordinate, ruffled species. A 5-coordinate complex is unlikely for a nickel corphinate in a coordinating solvent where two axial ligands would be readily available and formation of the 6-coordinate complex would be favored. Alternatively, the two species could be different ruffled species, for example, the two species obtained by a 90 ° rotation of the saddle structure resulting from S_4 ruffling and inversion of the half chair conformation of the hydroppyrrrolic rings (12,15).

Comparison of the F_{430} forms. Eschenmoser and coworkers have suggested that the sole difference between free and salt-extracted forms of F_{430} is a di-epimerization at positions 12 and 13 in which the hydrogen and carboxylic acid at each position exchange places (12). The structural change also occurs upon heating salt-extracted F_{430} or upon heat-extraction of F_{430} from methyl reductase. The 12,13-diepimer of salt-extracted F_{430} results in a corphinate structure that can undergo S_4 ruffling more readily than F_{430} for steric reasons. Ruffling affects axial ligation because it allows the central core of the macrocycle to contract about the nickel ion. Thus, salt-extracted F_{430} is more coordinatively unsaturated at the nickel ion as a result of its larger core size, and, therefore, has a higher affinity for axial ligands than free F_{430} which can easily ruffle.

The Raman results are in agreement with this structural interpretation. At room temperature salt-extracted F_{430} is mostly the 6-coordinate bis- H_2O complex, whereas free F_{430} at room temperature is 4-coordinate based on the Raman results. Further, the fraction of salt-extracted F_{430} that is 4-coordinate has a Raman spectrum that is distinct from 4-coordinate free F_{430} based on the Raman line frequencies. For example, the 1534- cm^{-1} line of salt-extracted F_{430} is at 1529 cm^{-1} for the diepimer. This difference is probably solely the result of the rearrangement of the substituents at the 12 and 13 positions of the corphinoid macrocycle.

Methyl reductase. The narrowing of the Raman lines of methyl reductase is most likely a result of greater homogeneity at the F_{430} site in the protein environment relative to the solution environment of the F_{430} forms. This indicates a very specific structure for the F_{430} site in the protein.

It is also clear from the large differences in frequency that the chromophore or its environment is unique in some way. The uniqueness may be a result of (1) a novel coordination geometry, (2) an unusual corphinate ligand conformation, or (3) a difference between the structure of the chromophore's peripheral substituents in the native protein and after salt extraction. We have observed that no oxygen, sulfur, or nitrogen ligand complex has come close to giving the frequencies observed for the two strong high frequency lines of methyl reductase. The separation of (1653 - 1575 =) 78 cm^{-1} for methyl reductase is however compatible with either 5- or 6-coordination although so far no ligand examined gives the high frequencies observed for these Raman lines. Thus, the second and third possibilities mentioned above should not be ruled out.

At 77 °K a form similar to the aqueous 6-coordinate form of salt-extracted F_{430} is beginning to make an appearance. At 4 °K the Ni X-ray absorption edge is

similar for methyl reductase and salt-extracted F_{430} (14). Thus, at 4 °K the salt-extracted F_{430} form may predominate in the protein.

Conclusions

The nickel-tetrapyrrole derivative in the methyl-reductase enzyme resides in a unique protein environment. We have not yet been able to find an equivalent structure for F_{430} in solution based on the Raman spectra. Nevertheless, the Raman data suggest that F_{430} in the protein has at least one axial ligand and occupies a site in the protein with a low degree of heterogeneity.

The free form of F_{430} shows evidence of ruffling in that multiple forms are observed in the low temperature Raman spectrum and the species cannot all be ascribed to axial ligand complexes. This low temperature behavior is also noted for the model nickel-corphinoid in non-coordinating solvents such as methylene chloride (5). On the other hand, salt-extracted F_{430} shows no evidence of ruffling, but displays higher affinity for axial ligands than the diepimer.

It is thought that the axial coordination sites of nickel may serve as a binding site for methyl-S-CoM or perhaps the methyl group that is reduced to methane in the reaction. Nickel corphinoids are known to have higher affinity for axial ligands than nickel porphyrins (15) and the unusual affinity may play a role in methane catalysis. The nickel corphinoids also exhibit different behavior in the photolysis of axial ligands than the nickel porphyrins (16). A Ni(I) intermediate may be involved in the reduction of the bound methyl group to methane (17).

Macrocycle flexibility may also play a role in methane catalysis as has been proposed for vitamin B₁₂ (Co-corrin) enzymes involved in biological methyl-transfer reactions (18,19). In these reactions an axial Co-CH₃ complex is known to be an intermediate. Efforts to further elucidate the roles of axial ligation and macrocycle flexibility in the catalytic properties of F_{430} in methyl reductase are continuing using resonance Raman and transient Raman spectroscopic techniques.

Acknowledgements

We thank Professor Albert Eschenmoser for kindly providing the model F_{430} complexes. This work performed at Sandia National Laboratories and supported by the U. S. Department of Energy Contract DE-AC04-76DP00789 and the Gas Research Institute Contract 5082-260-0767.

REFERENCES

1. Wolfe, R. S., Trends Biochem. Sci. 1985, 10, 396.
2. Hartzell, P. L.; Wolfe, R. S., Proc. Natl. Acad. Sci. USA 1986, 83, 6726.
3. Shiemke, A. K.; Scott, R. A.; Shelnutt, J. A., J. Am. Chem. Soc. 1987, submitted.
4. Shelnutt, J. A., J. Am. Chem. Soc. 1987, in press.
5. Shelnutt, J. A. unpublished results.
6. Ellefson, W. L.; Wolfe, R. S., J. Biol. Chem. 1981, 256, 4259.
7. Gunsalus, R. P.; Wolfe, R. S., J. Biol. Chem. 1980, 255, 1891.
8. Pfaltz, A.; Juan, B.; Fassler, A.; Eschenmoser, A.; Jaenchen, R.; Gilles, H. H.; Diekert, G.; Thauer, R. K., Helv. Chim. Acta 1982, 65, 828.
9. Fässler, A.; Pfaltz, A.; Kräutler, B.; Eschenmoser, A., J. Chem. Soc. Chem. Commun. 1984, 1365.
10. Shelnutt, J. A., J. Phys. Chem. 1983, 87, 605.
11. Livingston, D. A.; Pfaltz, A.; Schreiber, J.; Eschenmoser, A.; Ankel-Fuchs, D.; Moll, J.; Jaenchen, R.; Thauer, R. K., Helv. Chim. Acta 1984, 67, 334.

12. Pfaltz, A.; Livingston, D. A.; Jaun, B.; Diekert, G.; Thauer, R. K.; Eschenmoser, A., *Helv. Chim. Acta* 1985, **68**, 1338.
13. Eidsness, M. K.; Sullivan, R. J.; Schwartz, J. R.; Hartzell, P. L.; Wolfe, R. S.; Flank, A.-M.; Cramer, S. P.; Scott, R. A., *J. Am. Chem. Soc.* 1987, in press.
14. Shiemke, A. K.; Scott, R. A., unpublished results.
15. Eschenmoser, A., *Ann. N. Y. Acad. Sci.* 1986, **471**, 108.
16. Ondrias, M. R.; Finsen, E. W.; Crawford, B. A.; Shelnutz, J. A., *J. Am. Chem. Soc.* 1987, submitted.
17. Juan, B.; Pfaltz, A., *J. Chem. Soc. Chem. Commun.* 1986, 1327.
18. Wood, J. M. in "B₁₂" Vol 2, ed. Dolphin, D., Wiley: New York, 1982, p.151.
19. Geno, M. K.; Halpern, J., *J. Am. Chem. Soc.* 1987, **109**, 1238.

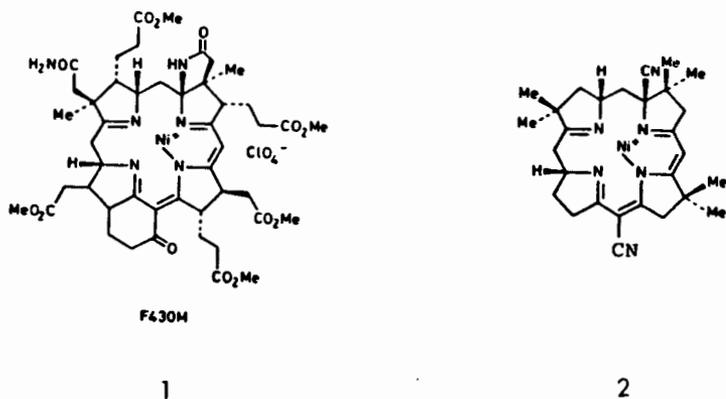


Fig. 1. Structures of F_{430} (1) and the model nickel-corphinoid derivative (2).

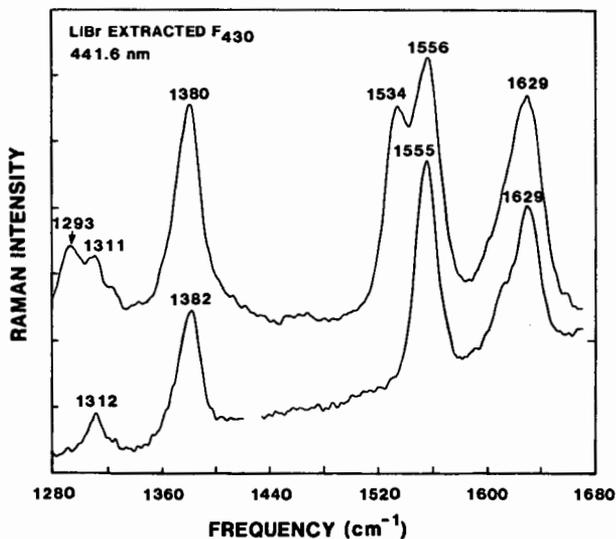


Fig. 2. Resonance Raman spectrum of salt-extracted F_{430} at room temperature (top) and at 77 °K (bottom) in aqueous 10 mM phosphate buffer.

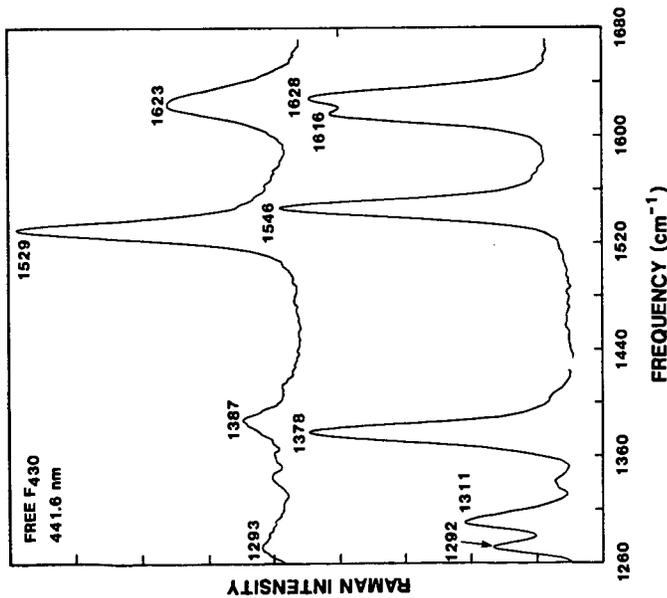


Figure 3. Resonance Raman spectrum of cytosol-free F₄₃₀ at room temperature (top) and at 77°K (bottom) in aqueous 10 nM phosphate buffer.

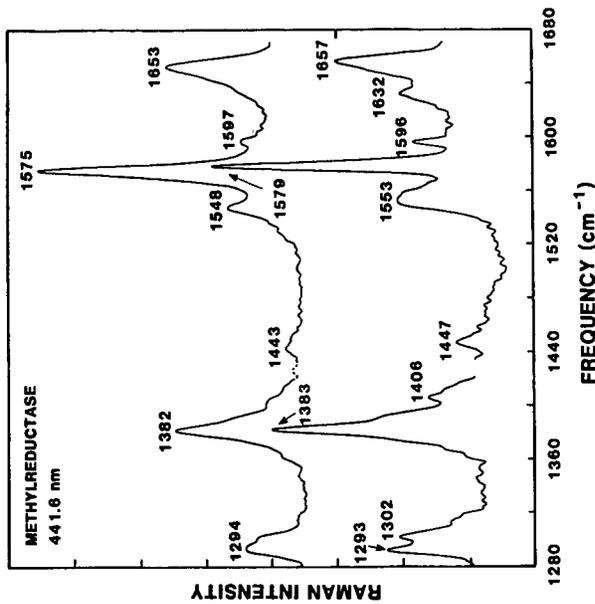


Figure 4. Resonance Raman spectrum of methyl reductase at room temperature (top) and at 77°K (bottom) in aqueous 10 nM phosphate buffer.